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with the lack of any removal mechanism makes them a very stable marker system. There is a fairly high certainty that if two individuals share an Alu at a specific site, they must share a common ancestor in which that insertion occurred (identity by descent) (15). Although the vast majority of Alu elements are found in common between humans and chimps, there are several hundred that were inserted recently enough to be polymorphic in the human population. These characteristics make Alu a stable genetic marker highly useful in forensic and anthropological studies of human DNA (15).

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AMELOGENIN

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Amelogenin is a critical component in the enamel of developing tooth germs (Fig. 1). The protein is thought to regulate the size and shape of crystals in the extracellular matrix. The endpoint of enamel maturation is the loss of amelogenin and replacement by highly ordered apatite crystals that make up 95%

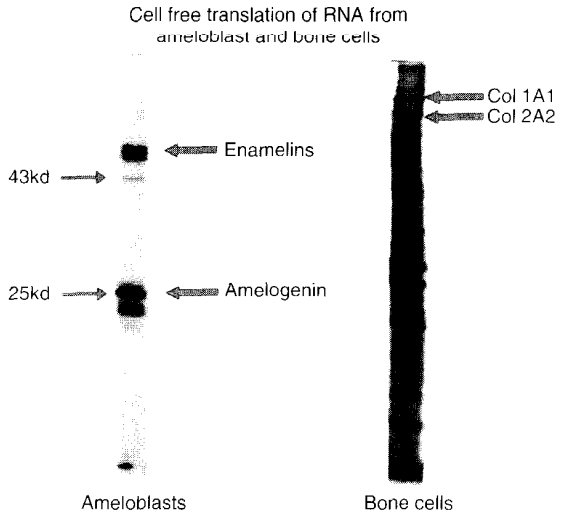

Figure 1. Comparison of mRNA translation products from ameloblasts and bone cells. RNA was extracted from ameloblasts and osteoblast-like cells and translated in a "cell free" rabbit reticulocyte system and separated on 10% denaturing acrylamide gels. The ameloblasts contain mRNA for two major classes of proteins, the amelogenins (~25 kD), and enamelins (~60 kD). Bone cells, in contrast, make a heterogeneous array of mRNA's, including the two chains of type I collagen. The clear difference in mRNA profiles and subsequent proteins produced may contribute to differences in the structural and functional properties of two mineralized tissues that they were derived from.

of the mature mineralized tissue. This unique feature makes enamel the hardest tissue in all vertebrates. The human amelogenin gene is approximately 8 kbp in length and is composed of 7 exons. Single copies of similar but not identical genes are transcribed from both the X- and Y-chromosomes, where the latter is 10-fold less active. In females, the gene is subject to X-chromosome inactivation. Mutations in the X-chromosome amelogenin gene have been described in patients with X-linked amelogenisis imperfecta (XAI), a syndrome characterized by hypomineralized or hypoplastic enamel with mineral crystals that are disorganized. Mutations range from large deletions of 5.0 kbp to single-point mutations leading to single amino acid substitutions. These observations provide evidence that the protein is essential for structural integrity of this unique tissue.

PROTEIN CHEMISTRY: STRUCTURE AND FUNCTION

Amelogenin is the most abundant protein in developing enamel and is produced by a highly specialized layer of epithelial cells in the developing tooth germ called ameloblasts (Fig. 1). It is highly hydrophylic, being composed primarily of proline, glutamine, leucine, and histidine residues and is highly conserved among species (1). It has no disulfide bonds. During enamel maturation, amelogenin undergoes extensive processing including posttranslational modification and enzymatic degradation, rendering the protein profile highly heterogeneous, when examined biochemically (2). The protein can self assemble to create nanosphere supramolecular structures in vivo, in a process that can be mimicked in vitro (3). Amelogenin is believed to bind directly to mineral and to regulate nucleation of crystal growth during enamel maturation. Bone is another tissue in the vertebrate that

contains mineral, but in contrast to enamel, it has less organized crystals that are 1,000 times smaller that those in enamel. This may be due to the fact that bone contains numerous distinct extracellular components such as type I collagen (Fig. 1) that may differentially regulate the quantity and quality of mineral found in bones and teeth.

MOLECULAR BIOLOGY: GENE STRUCTURE AND REGULATION

The human amelogenin gene was mapped to the X p22.1–22.3 and Y p11.2 chromosomes using somatic cell hybrids (4). Both X- and Y-loci generate alternatively spliced mRNA's providing additional theoretical basis for the observed protein heterogeneity. The largest human amelogenin mRNA is 845 b. Males and females have distinct amelogenin protein profiles (5). In the human, the longest gene product is 189–amino acid residues long and is rarely found.

Alternatively spliced mRNA's result in proteins that are 175–, 159–, 176–, and 145–amino acid residues; they are generated by "skipping" exon 4 or, 3 and 4 in the X-gene and exons 4 or 3, 4 and 5 in the Y-gene (6). The abundance of mRNA lacking exon 4 may be a function of its small size (64 nucleotides), which is thought to be unfavored in the splicing machinery.

Analysis of the X-chromosome promoter showed that 2,300 bp of DNA upstream from the start of transcription is required for its unique and highly tissue-specific expression (6). The identification of *trans*-acting (protein) factors that activate the gene have been hampered by the paucity of in vitro cell systems that express it. DNA silencing elements have additionally been uncovered and may contribute to the highly restricted activation of the gene during tooth development (7).

INHERITED DISEASE: X-LINKED AMELOGENESIS IMPERFECTA

XAI is a rare group of hereditary enamel defects characterized by vertical banding of the enamel in heterozygous females with a more uniform appearance in males. The enamel from patients with XAI is hypomineralized (with decreased enamel mineralization) and hypoplastic (with reduced enamel thickness) (8). Defective enamel formation in afflicted patients leads to discoloration and chipping away of protective enamel; extensive tooth restoration or extraction is often the major course of action. Females with XAI show alternating vertical areas of hypomature enamel with normal enamel presumably because of the Lyon effect.

To date, eight mutations in the human amelogenin gene have been identified in families with XAI (Table 1). The first mutation reported was a 5-kbp deletion leading to loss of exons 3 to 6 and part of 7 (9). DNA from other families showed a conversion of one base leading to the creation of a premature stop codon and, subsequently, a truncated protein was produced (10) (11) (12). In two separate cases, single base alterations were identified that resulted in a single amino acid conversion within the amelogenin protein. Specifically, Lench and Winter (12) found a C- to T-transition in exon 5 that converted a threonine residue to a isoleucine, whereas Collier and coworkers (13) found a family with a C- to A-transition in exon 6, converting a proline residue to a threonine. A general observation about these mutations is that they occur in regions of the protein that are highly conserved. Taken together, these findings provide strong evidence that amelogenin is critical for normal enamel formation in a process that is highly sensitive to changes in protein sequence and structure.

Table 1. Mutations in Human X-Chromosome Amelogenin Gene

Mutation	Exon Location	Protein Alteration	Reference
5.0 kb	Exon 3-6 and part of 7	18 N-terminal aa made (16 in signal)	[1]
CCCC>CCC	Exon 5	Loss of cytosine 74 N-terminal aa made	[2]
9-bp deletion	Exon 2	Loss of isoleucine, leucine and phenyalanine and new threonine made in signal peptide	[3]
CCCC>CCC	Exon 5	Nonsense same as [2]	[4]
C to T	Exon 5	Threonine to isoleucine	[5]
G to T	Exon 6	Lacks last 15 aa	[5]
C deleted	Exon 6	Lacks last 18 aa	[5]
C to A	Exon 6	Proline to threonine	[6]

Note: Summary of inherited mutations with in the X-linked human amelogenin gene. The nature and location of the mutation are shown in the first two columns. The theoretical alteration in the protein produced is listed next, where aa = amino acid. Primary references describing the mutations are listed in the last column.

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